

=> d his

(FILE 'HOME' ENTERED AT 10:32:07 ON 14 JUL 2003)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 10:32:19 ON
14 JUL 2003

L1 143135 S B2AR OR (BETA (3A) ADRENERGIC (3A) RECEPTOR?)
L2 1985936 S POLYMORPHISM? OR MUTATION? OR VARIANT? OR ALLELE
L3 5148 S L1 AND L2
L4 151 S L3 AND (491 OR 164 OR THR164? OR ILE164? OR C491? OR T491?)
L5 55 DUP REM L4 (96 DUPLICATES REMOVED)
L6 23 S L5 AND AGONIST
L7 23 S L5 AND (AGONIST OR SALMETEROL OR ALBUTEROL OR METAPROTERENOL)
L8 23 DUP REM L7 (0 DUPLICATES REMOVED)
L9 0 S L5 AND BONCHODI?
L10 5 S L5 AND BRONCHODI?
L11 5 DUP REM L10 (0 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 10:50:25 ON 14 JUL 2003

=>

L5 ANSWER 55 OF 55 MEDLINE DUPLICATE 35
ACCESSION NUMBER: 93192047 MEDLINE
DOCUMENT NUMBER: 93192047 PubMed ID: 8383511
TITLE: **Mutations** in the gene encoding for the
beta 2-adrenergic receptor in
normal and asthmatic subjects.
AUTHOR: Reihsaus E; Innis M; MacIntyre N; Liggett S B
CORPORATE SOURCE: Department of Medicine (Pulmonary), University of
Cincinnati College of Medicine, Ohio.
CONTRACT NUMBER: HL45967 (NHLBI)
SOURCE: AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY,
(1993 Mar) 8 (3) 334-9.
Journal code: 8917225. ISSN: 1044-1549.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930423
Last Updated on STN: 19990129
Entered Medline: 19930413
AB It has long been hypothesized that a defective **beta 2-adrenergic receptor (beta 2AR)** may be a pathogenic factor in bronchial asthma. We examined the gene encoding the beta 2AR to assess the frequency of **polymorphisms** in 51 patients with moderate to severe asthma and 56 normal subjects. Nine different point **mutations** were found in both heterozygous and homozygous forms at nucleic acid residues 46, 79, 100, 252, 491, 523, 1053, 1098, and 1239. No **mutations** resulting in large deletions or frame shifts were detected. Of these nine **polymorphisms**, four were found to cause changes in the encoded amino acids at residues 16, 27, 34, and 164. The most frequent **polymorphisms** were arginine 16 to glycine (Arg16-->Gly) and glutamine 27 to glutamic acid (Gln27-->Glu). The other two **polymorphisms**, valine 34 to methionine, and threonine 164 to isoleucine, occurred in only four subjects. The incidence of beta 2AR homozygous **polymorphisms** was no greater in asthmatic patients as compared with controls (Arg16-->Gly: 53% versus 59%, Gln27-->Glu: 24% versus 29%, respectively; P = NS). Some subjects were found to have both of these **polymorphisms** simultaneously, but there was no difference in incidence between the two groups, with 23% of asthmatics and 28% of normal subjects being homozygous for both **polymorphisms**. The apparently normal subjects with both **polymorphisms** did not have subclinical hyperreactive airways disease as determined by methacholine challenge testing. In the asthma group, one **mutation** (Arg16-->Gly) identified a subset of patients with a distinct clinical profile. (ABSTRACT TRUNCATED AT 250 WORDS)

L8 ANSWER 1 OF 23 MEDLINE
ACCESSION NUMBER: 2003125791 MEDLINE
DOCUMENT NUMBER: 22526722 PubMed ID: 12525504
TITLE: Hierarchy of polymorphic variation and desensitization permutations relative to beta 1- and **beta** 2-
adrenergic receptor signaling.
AUTHOR: Rathz Deborah A; Gregory Kimberly N; Fang Ying; Brown Kari M; Liggett Stephen B
CORPORATE SOURCE: Department of Pharmacology, University of Cincinnati College of Medicine, Ohio 45267-0564, USA.
CONTRACT NUMBER: GM61376 (NIGMS)
HD07463 (NICHD)
HL22619 (NHLBI)
HL52318 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2003 Mar 21) 278 (12) 10784-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 20030318
Last Updated on STN: 20030506
Entered Medline: 20030505
AB Agonist-promoted desensitization of G-protein-coupled receptors results in partial uncoupling of receptor from cognate G-protein, a process that provides for rapid adaptation to the signaling environment. This property plays important roles in physiologic and pathologic processes as well as therapeutic efficacy. However, coupling is also influenced by polymorphic variation, but the relative impact of these two mechanisms on signal transduction is not known. To determine this we utilized recombinant cells expressing the human **beta**(1)-
adrenergic receptor (**beta**(1)AR) or a gain-of-function polymorphic **variant** (**beta**(1)AR-Arg(389)), and the **beta**(2)-**adrenergic receptor** (**beta**(2)AR) or a loss-of-function polymorphic receptor (**beta**(2)AR-Ile(164)). Adenylyl cyclase activities were determined with multiple permutations of the possible states of the receptor: genotype, basal, or **agonist** stimulated and with or without **agonist** pre-exposure. For the **beta**(1)AR, the enhanced function of the Arg(389) receptor underwent less **agonist**-promoted desensitization compared with its allelic counterpart. Indeed, the effect of polymorphic variation on absolute adenylyl cyclase activities was such that desensitized **beta**(1)AR-Arg(389) signaling was equivalent to non-desensitized wild-type **beta**(1)AR; that is, the genetic component had as much impact as desensitization on receptor coupling. In contrast, the enhanced signaling of wild-type **beta**(2)AR underwent less desensitization compared with **beta**(2)AR-Ile(164), thus the heterogeneity in absolute signaling was markedly broadened by this polymorphism. Inverse **agonist** function was not affected by polymorphisms of either subtype. A general model is proposed whereby up to 10 levels of signaling by G-protein-coupled receptors can be present based on the influences of desensitization and genetic variation on coupling.

L8 ANSWER 2 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2003038498 EMBASE
TITLE: Influence of the **thr164ile polymorphism** in the **.beta.(2)-adrenoceptor** on the effects of **.beta.-adrenoceptor agonists** on human lung mast cells.
AUTHOR: Kay L.J.; Chong L.K.; Rostami-Hodjegan A.; Peachell P.T.

CORPORATE SOURCE: P.T. Peachell, Molec. Pharmacol./Pharmacogenetics, University of Sheffield, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF, United Kingdom.
p.t.peachell@shef.ac.uk

SOURCE: International Immunopharmacology, (2003) 3/1 (91-95).
Refs: 17
ISSN: 1567-5769 CODEN: IINMBA

PUBLISHER IDENT.: S 1567-5769(02)00217-5

COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 022 Human Genetics
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB We have examined the influence of the **thr164ile polymorphism** in the .beta.(2)-adrenoceptor on the ability of the .beta.-adrenoceptor **agonists**, isoprenaline and salbutamol, to stabilise human lung mast cells. A total of 124 mast cell preparations were genotyped and, of these, 120 were found to be homozygous (**thr164thr**) at position **164** of the .beta.(2)-adrenoceptor and 4 were heterozygous (**thr164ile**). None of the preparations was homozygous for ile at position **164**. In these preparations, the effects of isoprenaline and salbutamol on the IgE-mediated release of histamine from mast cells were studied. Both isoprenaline and salbutamol inhibited histamine release in a concentration-dependent manner. Average inhibitory potencies for both **agonists**, as assessed by pD(2) values, were higher in homozygous than in heterozygous preparations. For isoprenaline, this difference was statistically significant (P<0.005), whereas for salbutamol, it was not (P=0.21). These data suggest that the **thr164ile polymorphism** in the .beta.(2)-adrenoceptor may influence the extent to which certain .beta.-adrenoceptor **agonists** inhibit the responses of mast cells. .COPYRGT. 2002 Elsevier Science B.V. All rights reserved.

L8 ANSWER 3 OF 23 MEDLINE

ACCESSION NUMBER: 2002444594 MEDLINE

DOCUMENT NUMBER: 22191661 PubMed ID: 12202992

TITLE: SNP genotyping in the **beta(2)-adrenergic receptor** by electronic microchip assay, DHPLC, and direct sequencing.

AUTHOR: Yoshida Nobuyo; Nishimaki Yuko; Sugiyama Masahide; Abe Takashi; Tatsumi Taiga; Tanoue Akito; Hirasawa Akira; Tsujimoto Gozoh

CORPORATE SOURCE: Department of Molecular, Cell Pharmacology, National Research Institute for Child Health and Development, 3-35-31 Taishido, Setagaya-ku, Tokyo 154-8567, Japan.

SOURCE: JOURNAL OF HUMAN GENETICS, (2002) 47 (9) 500-3.
Journal code: 9808008. ISSN: 1434-5161.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200212

ENTRY DATE: Entered STN: 20020831
Last Updated on STN: 20021217
Entered Medline: 20021206

AB The **beta(2)-adrenergic receptor** (**beta2AR**) is the key target for the **beta(2)-agonist** drugs used for bronchodilation in asthma and chronic obstructive pulmonary disease. To detect four SNPs with amino acid variations at positions -47T/C (CysBUP19Arg), 46A/G (Gly16Arg), 79C/G (Gln27Glu), and 491C/T (**Thr164Ile**) in the beta 2AR gene, we used the electronic microchip

assay, denaturing high-performance liquid chromatography (DHPLC), and direct sequencing. Genomic DNA samples were obtained from the blood of 84 Japanese healthy volunteers. The agreement rates of the first data set with the final data (**allele** calls) were 99.7% (332/333), 99.2% (246/248), and 96.7% (329/340). The percentages of no **allele** designation (ND) were 2.06% (7/340), 2.75% (7/255), and 0.00% (0/340) for the electronic microchip assay, DHPLC, and direct sequencing, respectively. Furthermore, we found three samples that had a novel haplotype.

L8 ANSWER 4 OF 23 MEDLINE
ACCESSION NUMBER: 2002394110 MEDLINE
DOCUMENT NUMBER: 22138213 PubMed ID: 12142724
TITLE: beta2 adrenoceptor gene **polymorphisms** in cystic fibrosis lung disease.
COMMENT: Comment in: Pharmacogenetics. 2002 Jul;12(5):345-6
AUTHOR: Buscher Rainer; Eilmes Katrin Jennifer; Grasemann Hartmut;
Torres Brian; Knauer Nicola; Sroka Karin; Insel Paul A;
Ratjen Felix
CORPORATE SOURCE: Children's Hospital, University of Essen, Germany..
rainer.buescher@uni-essen.de
SOURCE: PHARMACOGENETICS, (2002 Jul) 12 (5) 347-53.
Journal code: 9211735. ISSN: 0960-314X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200303
ENTRY DATE: Entered STN: 20020727
Last Updated on STN: 20030314
Entered Medline: 20030313

AB The cystic fibrosis membrane conductance regulator can be activated through beta2-adrenoceptor (beta2AR) stimulation. We tested the hypothesis that coding sequence **polymorphisms** in the beta2AR gene contribute to the disease state in patients with cystic fibrosis. The Arg16Gly, Gln27Glu, and **Thr164Ile** beta2AR **polymorphisms** were studied by specific polymerase chain reaction and restriction fragment length **polymorphism** analysis in 126 cystic fibrosis patients. Forced expiratory volume in 1 s was significantly ($P < 0.05$) reduced in cystic fibrosis patients carrying the Gly16 **allele** in either homozygous or heterozygous form (Gly16Gly + Arg16Gly) compared to patients homozygous for the Arg16 **allele** ($60.3 +/- 3.5\%$ versus $75.7 +/- 4.9\%$ predicted). Similarly, forced vital capacity and flows at lower lung volumes were significantly ($P < 0.05$ and $P < 0.01$) lower in cystic fibrosis patients carrying the Gly16 **allele**. In addition, the Gly16 **allele** was associated with a greater 5 year decline in pulmonary function ($P < 0.01$). Bronchodilator responses to **albuterol** were not significantly different between the groups. The **Thr164Ile variant** was found in four patients; these patients had markedly reduced pulmonary function. Isoproterenol-stimulated cyclic AMP formation was significantly blunted in cystic fibrosis patients carrying either the Gly16 **allele** or **Thr164Ile** genotype compared to cystic fibrosis patients homozygous for the respective Arg16 **alleles**. These data provide the first evidence suggesting that **polymorphisms** of the beta2AR gene contribute to clinical severity and disease progression in cystic fibrosis.

L8 ANSWER 5 OF 23 MEDLINE
ACCESSION NUMBER: 2002341435 MEDLINE
DOCUMENT NUMBER: 22078897 PubMed ID: 12083943
TITLE: Clinical importance of beta-adrenoceptor **polymorphisms** in cardiovascular disease.
AUTHOR: McNamara Dennis M; MacGowan Guy A; London Barry

CORPORATE SOURCE: Department of Medicine, Heart Failure Section, University of Pittsburgh, Pittsburgh, Pennsylvania 15213-2582, USA..
mcnamaradm@msx.upm.edu

CONTRACT NUMBER: HL 03826 (NHLBI)
HL 62300 (NHLBI)
HL 69912 (NHLBI)

SOURCE: Am J Pharmacogenomics, (2002) 2 (2) 73-8. Ref: 35
Journal code: 100967746. ISSN: 1175-2203.

PUB. COUNTRY: New Zealand

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200212

ENTRY DATE: Entered STN: 20020627
Last Updated on STN: 20021227
Entered Medline: 20021226

AB beta-Adrenoceptor antagonists play an important role in the treatment of cardiovascular disease and have been used for three decades in the treatment of hypertension and ischemic heart disease. More recently they have been demonstrated to improve survival in patients with mild to moderate congestive heart failure. The beneficial effects of beta-adrenoceptor antagonists stems from their ability to limit the deleterious effects of adrenergic stimulation, which in the cardiovascular system is primarily transmitted through two subclasses of receptor, beta(1) and beta(2). The advances of the Human Genome Project have led to an increased appreciation that variations in genetic background may underlie a substantial portion of the clinical heterogeneity apparent in cardiovascular disease. This review examines the molecular, functional, and clinical significance of the most common **polymorphisms** of the beta(1) and beta(2)-adrenoceptors. Initial research in adrenoceptor variation focused on the beta(2)-adrenoceptor. Three common **polymorphisms** appear to influence receptor function: Arg16-->Gly, Glu(27)-->Gln, and Thr(164)-->Ile. In in vitro studies of **agonist** stimulation, Gly(16) receptors demonstrate enhanced downregulation, while Glu(27) **variants** are resistant to downregulation. There is much controversy and conflict among various clinical studies regarding the effect of these **variants** on vasoreactivity and hypertensive risk. The Ile(164) **variant** demonstrates decreased responsiveness to **agonist** activity both in vitro and in animal models. In studies of patients with congestive heart failure, this **variant** has been associated with poor functional capacity and decreased survival. More recent investigations have focused on the two common **polymorphisms** of the beta(1)-adrenoceptor: Ser(49)-->Gly, and Arg(389)-->Gly. In vitro studies of Arg(389) receptors demonstrate a gain of function, as **agonist** stimulation results in significantly higher intracellular levels of cyclic adenosine monophosphate when compared with the Gly(389) **variant**. Consistent with the in vitro data, clinical studies demonstrate increased responsiveness to beta-**agonist** stimulation, and an increased risk of hypertension among Arg(389) homozygotes. Further investigation of the clinical implications of these common **variants** of beta(1)- and beta(2)-adrenoceptors are needed. Importantly, the pharmacogenetic impact of these **variants** on the effectiveness of beta-adrenergic blockade remains unknown.

L8 ANSWER 6 OF 23 MEDLINE
ACCESSION NUMBER: 2002106444 MEDLINE
DOCUMENT NUMBER: 21827057 PubMed ID: 11836685
TITLE: Association between the genetic **polymorphisms** of beta2-adrenergic receptor gene and the asthma susceptibility and clinical phenotypes in a Chinese population.

AUTHOR: Fu Jin; Chen Hong; Hu Liangping; Zhang Huiqin; Ma Yu; Chen Yuzhi
CORPORATE SOURCE: Department of Biochemistry and Immunity, Capital Institute of Pediatrics, Beijing, 100020 P. R. China..
hchen@public.bta.net.cn
SOURCE: CHUNG-HUA I HSUEH I CHUAN HSUEH TSA CHIH, (2002 Feb) 19 (1)
41-5.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 20020212
Last Updated on STN: 20020406
Entered Medline: 20020405

AB OBJECTIVE: To determine whether genetic **polymorphisms** of beta2-adrenergic receptor gene affect asthma susceptibility and play a role in disease regulation. METHODS: One hundred and sixty-six unrelated childhood asthma cases and 32 families with 192 samples were studied. The **polymorphisms** at amino acid positions 16, 27, 164 and nucleic acid residue 523 were genotyped by polymerase chain reaction-restriction endonuclease digestion. RESULTS: The amino acid 164 Thr/Ile **variant** was seen only in the heterozygote form, and it occurred with a frequency of 3% which is similar to the published results among Caucasians. There were significant differences in the **allele** frequencies of the other 3 **polymorphisms** between Chinese and the published results among Caucasians ($P<0.001$). No **polymorphism** was found to be associated with total serum IgE, the number of positive prick skin test and FEV1. No significant association was noted between either the arginine-glycine 16 or the glutamine-glutamate 27 **polymorphisms** and the airway responsiveness to beta2-agonists in childhood asthma cases. CONCLUSION: In this study population, the authors were unable to confirm that the **polymorphism** of beta2-adrenergic receptor gene is a crucial factor of the susceptibility to asthma and a major genetic determinant of different clinical status.

L8 ANSWER 7 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2003:78839 BIOSIS
DOCUMENT NUMBER: PREV200300078839
TITLE: Genetic modulation of human cardiac performance by Ile-164 polymorphism of beta-2 adrenergic receptor.
AUTHOR(S): Barbato, Emanuele (1); Penicka, Martin (1); Delrue, Leen (1); Vanderheyden, Marc (1); Wijns, William (1); Heyndrickx, Guy (1); Goethals, Marc (1); de Bruyne, Bernard (1); Bartunek, Jozef (1)
CORPORATE SOURCE: (1) CV Ctr, Aalst, Aalst, Belgium Belgium
SOURCE: Circulation, (November 5 2002) Vol. 106, No. 19 Supplement, pp. II.327-II.328. print.
Meeting Info.: Abstracts from Scientific Sessions Chicago, IL, USA November 17-20, 2002 American Heart Association . ISSN: 0009-7322.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 8 OF 23 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2001:821967 SCISEARCH
THE GENUINE ARTICLE: 481CW
TITLE: Constitutive activation of the mu opioid receptor by mutation of D3.49(164), but not D3.32(147): D3.49(164) is critical for stabilization of the inactive form of the receptor and for

AUTHOR: its expression
Li J; Huang P; Chen C G; de Riel J K; Weinstein H;
Liu-Chen L Y (Reprint)

CORPORATE SOURCE: Temple Univ, Sch Med, Dept Pharmacol, 3420 N Broad St,
Philadelphia, PA 19140 USA (Reprint); Temple Univ, Sch
Med, Dept Pharmacol, Philadelphia, PA 19140 USA; Temple
Univ, Sch Med, Ctr Subst Abuse Res, Philadelphia, PA 19140
USA; Temple Univ, Sch Med, Fels Inst Mol Biol & Canc Res,
Philadelphia, PA 19140 USA; CUNY Mt Sinai Sch Med, Dept
Physiol & Biophys, New York, NY 10029 USA

COUNTRY OF AUTHOR: USA

SOURCE: BIOCHEMISTRY, (9 OCT 2001) Vol. 40, No. 40, pp.
12039-12050.

Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW,
WASHINGTON, DC 20036 USA.

ISSN: 0006-2960.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 61

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The roles of conserved aspartates in the third transmembrane domain of the rat mu opioid receptor (RMOR) were explored with mutations of D3.32(147) and D3.49(164). D3.49(164) in the highly conserved DRY motif was mutated to 13 amino acids. Except for the D3.49(164)E mutant, each mutant displayed little or no detectable [³H]diprenorphine binding, and pretreatment with naloxone greatly enhanced binding. D3.49(164)H, -Q, -Y, -M, and -E mutants were further studied. D3.32(147) was substituted with A or N. All seven mutants exhibited similar binding affinities for the antagonist [³H]diprenorphine as the wildtype. The D3.49(164)H, -Q, -Y, and -M mutants, but not the D3.49(164)E and D3.32(147) mutants, exhibited enhanced basal [³⁵S]-GTP gammaS binding which was comparable to the maximally activated level of the wild-type and was related to expression levels. Naloxone, naltrexone, and naloxone methiodide significantly inhibited the basal [³⁵S]-GTP gammaS binding of the D3.49(164) mutants, indicating inverse agonist activities. Treatment of the D3.49(164)Y mutant with pertussis toxin greatly reduced the basal [³⁵S]-GTP gammaS binding, demonstrating constitutive activation of G alpha (i)/alpha (o). The D3.49(164)H, -Y, -M, and -Q mutants had higher affinities for DAMGO than the wild-type, which were not significantly lowered by GTP gammaS. Thus, mutation of D3.49(164) to H, Y, M, or Q in RMOR resulted in receptor assuming activated conformations. In contrast, the D3.49(164)E mutant displayed significantly lower basal [³⁵S]-GTP,IS binding and reduced affinity for DAMGO. Upon incubation of membranes at 37 degreesC, the constitutively active D3.49(164)Y mutant was structurally less stable, whereas the inactivated D3.49(164)E mutant was more stable, than the wild-type. Computational simulations showed that the E3.49 side chain interacted strongly with the conserved R3.50 in the DRY motif and stabilized the inactive form of the receptor. Taken together, these results indicate that D3.49 plays an important role in constraining the receptor in inactive conformations.

L8 ANSWER 9 OF 23 MEDLINE

ACCESSION NUMBER: 2002059980 MEDLINE

DOCUMENT NUMBER: 21642957 PubMed ID: 11785682

TITLE: Beta2-adrenergic receptor genotype-related changes in cAMP levels in peripheral blood mononuclear cells after multiple-dose oral procaterol.

AUTHOR: Makimoto H; Sakaeda T; Nishiguchi K; Kita T; Sakai T;
Komada F; Okumura K

CORPORATE SOURCE: Department of Hospital Pharmacy, School of Medicine, Kobe University, Japan.

SOURCE: PHARMACEUTICAL RESEARCH, (2001 Dec) 18 (12) 1651-4.

PUB. COUNTRY: Journal code: 8406521. ISSN: 0724-8741.
United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020807
Entered Medline: 20020806

AB PURPOSE: To evaluate the beta2-adrenergic receptor (beta2AR) genotype frequency in the Japanese population and the relationship between beta2AR genotype at amino acid position 16 (beta2AR-16) and desensitization to beta2-agonist ex vivo. METHODS: The beta2AR genotypes at amino acid positions 16, 27, and 164 of 92 healthy Japanese subjects were determined by polymerase chain reaction-restriction fragment-length polymorphism. The relationship between the beta2AR-16 genotype and the desensitization to beta2-agonist was examined in 10 male subjects ex vivo. Procaterol tablet (HCl salt, 50 microg, Meptin) was given orally for 5 days, and peripheral blood was obtained before and after 5 days of consecutive medications followed by the assessment of the intracellular cAMP levels in peripheral blood mononuclear cells after incubation with or without procaterol hydrochloride (0-1000 ng/mL). RESULTS: Allele frequency was Arg16:Gly16 = 46%:54%, Gln27:Glu27 = 92%:8%, and Thr164:Ile164 = 100%:0%, respectively. The cAMP levels were increased by incubation with procaterol hydrochloride, and the increase was suppressed after 5 days of consecutive medications. The suppression was more significant in the homozygote for Gly16 than the homozygote for Arg16. CONCLUSIONS: The desensitization to beta2-agonist was associated more frequently with the mutation at beta2AR-16 (Gly16).

L8 ANSWER 10 OF 23 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2001:904846 SCISEARCH
THE GENUINE ARTICLE: 489HE
TITLE: Inverse agonist up-regulates the constitutively active D3.49(164)Q mutant of the rat mu-opioid receptor by stabilizing the structure and blocking constitutive internalization and down-regulation
AUTHOR: Li J; Chen C G; Huang P; Liu-Chen L Y (Reprint)
CORPORATE SOURCE: Temple Univ, Sch Med, Dept Pharmacol, 3420 N Broad St, Philadelphia, PA 19140 USA (Reprint); Temple Univ, Sch Med, Dept Pharmacol, Philadelphia, PA 19140 USA
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR PHARMACOLOGY, (NOV 2001) Vol. 60, No. 5, pp. 1064-1075.
' Publisher: AMER SOC PHARMACOLOGY EXPERIMENTAL THERAPEUTICS 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.
ISSN: 0026-895X.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 45

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We demonstrated previously that D3.49(164) mutations resulted in constitutive activation of the rat mu-opioid receptor and abolished receptor expression unless cells were pretreated with naloxone, an inverse agonist. In this study, we investigated the properties of the D3.49(164)Q mutant and the mechanisms underlying the effect of naloxone. Naloxone pretreatment upregulated [³H]diprenorphine binding and protein expression of the D3.49(164)Q mutant in a time- and dose-dependent manner without affecting its mRNA level. After naloxone removal, binding and protein expression of the mutant declined with time with no effect on its mRNA level. Naloxone methiodide (a quaternary ammonium analog) caused a maximal up-regulation about 50% of the naloxone effect, indicating that naloxone acts

extracellularly and intracellularly. Expression of the mutant was enhanced by inverse **agonists**, a neutral antagonist, and **agonists**, with inverse **agonists** being most effective. In membranes, the mutant was structurally less stable than the wild type upon incubation at 37 degreesC, and naloxone and [D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin stabilized the mutant. Coexpression of the dominant-negative mutants GRK2-K220R, arrestin-2(319-418), dynamin I-K44A, rab5A-N133I or rab7-N125I partially prevented the decline in binding of the mutant after naloxone removal. Chloroquine or proteasome inhibitor I reduced the down-regulation of the mutant. These results indicate that the D3.49(164)Q mutant is constitutively internalized via G protein coupled-receptor kinase-, arrestin-2-, dynamin-, rab5-, and rab7-dependent pathways and probably trafficked through early and late endosomes into lysosomes and degraded by lysosomes and proteasomes. Naloxone up-regulates the D3.49(164)Q mutant by stabilizing the mutant protein and blocking its constitutive internalization and down-regulation. To the best of our knowledge, this represents the first comprehensive analysis of the mechanisms involved in up-regulation of constitutively active mutants by an inverse **agonist**.

L8 ANSWER 11 OF 23 MEDLINE
ACCESSION NUMBER: 2001265446 MEDLINE
DOCUMENT NUMBER: 21127924 PubMed ID: 11222464
TITLE: Blunted cardiac responses to receptor activation in subjects with **Thr164Ile** beta(2)-adrenoceptors.
AUTHOR: Brodde O E; Buscher R; Tellkamp R; Radke J; Dhein S; Insel P A
CORPORATE SOURCE: Department of Pharmacology, University of Halle, Germany.
CONTRACT NUMBER: HL58120 (NHLBI)
SOURCE: CIRCULATION, (2001 Feb 27) 103 (8) 1048-50.
Journal code: 0147763. ISSN: 1524-4539.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010618
Last Updated on STN: 20010618
Entered Medline: 20010614

AB BACKGROUND: Recent evidence indicates that certain genotypes of beta(2)-adrenoceptors (AR) may indicate an increased risk of cardiovascular disease or an increased rate of disease progression. Of particular importance, the **Thr164Ile polymorphism**, which is found in approximately 4% of humans, shows decreased receptor signaling, blunted cardiac response when expressed in transgenic mice, and is associated with a decreased survival rate in patients with congestive heart failure. METHODS AND RESULTS: In this study, we compared functional activity, ie, chronotropic (heart rate increases) and inotropic (duration of the electromechanical systole) responses to intravenously administered **terbutaline**, in 6 subjects (4 women and 2 men) who were heterozygous for **Thr164Ile** with the responses in 12 volunteers (6 women and 6 men) who were homozygous for wild-type (WT) beta(2)-AR (ie, Arg16, Gln27, and **Thr164**). The beta(2)AR **polymorphism** significantly affected the dose-response curves for **terbutaline**-induced inotropic and chronotropic responses: compared with WT individuals, subjects with the **Thr164Ile** receptor had substantial blunting in maximal increases in heart rate (WT, 29.7+/-3.9 beats/min; **Ile164**, 20.7+/-1.9 beats/min; P:=0.016) and a shortening of the duration of electromechanical systole (WT, 51.9+/-4.5 ms; **Ile164**, 37.9+/-4.6 ms; P:=0.02). CONCLUSIONS: These data show that humans with the **Ile164** genotype show blunted cardiac beta(2)-AR responsiveness, which may help explain the decreased survival of patients with this genotype in the setting of congestive heart failure.

L8 ANSWER 12 OF 23 MEDLINE
ACCESSION NUMBER: 2001401205 MEDLINE
DOCUMENT NUMBER: 21322120 PubMed ID: 11429395
TITLE: The effect of the beta(2) adrenoceptor gene
Thr164Ile polymorphism on human adipose
tissue lipolytic function.
AUTHOR: Hoffstedt J; Iliadou A; Pedersen N L; Schalling M; Arner P
CORPORATE SOURCE: Department of Medicine, Karolinska Institute and Research
Center at CME, Huddinge Hospital, 141 86 Huddinge, Sweden.
SOURCE: BRITISH JOURNAL OF PHARMACOLOGY, (2001 Jul) 133 (5) 708-12.
Journal code: 7502536. ISSN: 0007-1188.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010813
Last Updated on STN: 20010813
Entered Medline: 20010809

AB A rare beta(2)-adrenoceptor gene polymorphism, Thr164Ile, has been described that impairs receptor function when transfected into cell lines. We investigated whether the polymorphism influences native receptor function by studying lipolysis in freshly isolated subcutaneous fat cells from 236 apparently healthy subjects. Twelve subjects were heterozygous for the 164Ile variant. The fat cells of Ile carriers displayed a 6 fold increase ($P=0.02$) in the lipolytic EC(50) of terbutaline (a selective beta(2)-adrenoceptor agonist), but no change in the lipolytic action of dobutamine (a selective beta(1)-adrenoceptor agonist), compared with the Thr carriers. Maximum adrenoceptor agonist stimulated lipolysis did not differ between Thr and Ile carriers. The influence of two other polymorphisms (Arg16Gly and Gln27Glu) in the beta(2)-adrenoceptor gene was considered. Six 164Ile carriers also carried the 16Gly and 27Glu alleles. The latter combination occurred among 105 of the 164Thr carriers. For the 16Gly27Glu subgroup, the EC(50) of terbutaline was about 10 fold higher in 164Ile as than in 164Thr carriers ($P=0.02$) but there was no difference between genotypes in maximum terbutaline action. There was no difference between groups in dobutamine action. In conclusion, the 164Ile variant of the beta(2)-adrenoceptor is associated with a decreased native adipocyte receptor function, as evidenced by a marked increase in the half maximal effective concentration of the lipolytic action of a selective beta(2)-adrenoceptor agonist. This suggests that genetic variance in the beta(2)-adrenoceptor gene might be important for catecholamine function in humans, at least as far as adipocyte lipolysis is concerned.

L8 ANSWER 13 OF 23 MEDLINE
ACCESSION NUMBER: 2002341412 MEDLINE
DOCUMENT NUMBER: 22078878 PubMed ID: 12083965
TITLE: Genetic variation of the beta(2)-adrenoceptor: its
functional and clinical importance in bronchial asthma.
AUTHOR: Taylor D R; Kennedy M A
CORPORATE SOURCE: Department of Medicine, Dunedin School of Medicine,
University of Otago, Dunedin, New Zealand..
robin.taylor@stonebow.otago.ac.nz
SOURCE: Am J Pharmacogenomics, (2001) 1 (3) 165-74. Ref: 62
Journal code: 100967746. ISSN: 1175-2203.
PUB. COUNTRY: New Zealand
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020627
Last Updated on STN: 20020725
Entered Medline: 20020724

AB Asthma is a polygenic disease for which no clear genotype-phenotype relationships have emerged. In contrast, although not associated with the diagnosis of asthma per se, **variant** forms of the beta(2)-adrenoceptor (beta2-AR) gene (ADRB2) display functional effects that may be clinically relevant. Single nucleotide **polymorphisms** (SNPs) of ADRB2 are common and result in amino acid substitutions at positions 16, 27, and 164 of the receptor as well as position 19 of its 5' upstream peptide. These SNPs influence receptor function *in vitro*, although evidence regarding exact relationships is conflicting. This has raised the possibility that phenotypes such as bronchial hyper-responsiveness (BHR) and responses to (beta2)-**agonist** drugs may be genetically determined. To date, no unequivocal relationships between SNPs and phenotype have been identified. In some studies the Gly(16) **allele** has been associated with increased BHR and asthma severity. In others, the Arg(16) **allele** has been shown to determine acute bronchodilator response and adverse events during long term beta(2)-**agonist** therapy. The latter may provide the basis for clinical application of this new knowledge. More recently, a small number of frequently occurring, functionally relevant ADRB2 haplotype pairs have been confirmed. These combinations of **alleles** may be more important in determining genotype/phenotype relationships than individual SNPs, and may explain why earlier investigations have yielded contrasting results. Future studies will be required to clarify the pharmacodynamic effects of ADRB2haplotypes both *in vitro* and *in vivo*.

L8 ANSWER 14 OF 23 MEDLINE
ACCESSION NUMBER: 2001470892 MEDLINE
DOCUMENT NUMBER: 21407872 PubMed ID: 11516429
TITLE: The Ile164 beta(2)-adrenoceptor polymorphism alters salmeterol exosite binding and conventional agonist coupling to G(s).

AUTHOR: Green S A; Rathz D A; Schuster A J; Liggett S B
CORPORATE SOURCE: Department of Medicine, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Room G062, Cincinnati, OH 45267-0564, USA.

CONTRACT NUMBER: GM61376 (NIGMS)
HD07463 (NICHD)
HL45967 (NHLBI)

SOURCE: EUROPEAN JOURNAL OF PHARMACOLOGY, (2001 Jun 15) 421 (3) 141-7.

Journal code: 1254354. ISSN: 0014-2999.

PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200109

ENTRY DATE: Entered STN: 20010823
Last Updated on STN: 20010910
Entered Medline: 20010906

AB beta(2)-adrenoceptors (beta(2)AR) are polymorphic at amino acid 164 (Thr or Ile) of the fourth transmembrane domain. In transfected fibroblasts, six **agonists** commonly used in the treatment of bronchospasm were studied. Isoproterenol, **albuterol**, **metaproterenol**, **terbutaline**, **formoterol**, and **salmeterol** displayed decreased binding affinities ($K(i)_s$ were 1.2-3.0-fold higher) and a significant degree of impaired maximal stimulation of adenylyl cyclase (approximately 40%), was observed with all **agonists** for the Ile164 receptor. The ratios of

signal transduction efficiencies (Tau function, **Ile164/Thr164**) varied from a low of 0.17 for **terbutaline** to 0.49 for **salmeterol**. In addition, **Ile164** bound **salmeterol** at the exosite, as delineated in perfusion washout studies, at a decreased level (31+/-4.8% vs. 49+/-4.4% retained **salmeterol**, respectively, $P=0.02$). In cAMP production studies under perfusion conditions, this decreased exosite binding caused a approximately 50% decrease in the duration of action of **salmeterol** at **Ile164** ($t(1/2)=21.0+/-3.6$ vs. $46.8+/-4.1$ min for **Thr164**, $P=0.001$). The durations of action for **isoproterenol** and **formoterol** under similar perfusion conditions were not different between the two receptors. These *in vitro* results indicate the **Ile164** polymorphic receptor represents a pharmacogenetic locus for the most commonly utilized agonists in the treatment of asthma with a unique phenotype for **salmeterol**.

L8 ANSWER 15 OF 23 MEDLINE

ACCESSION NUMBER: 2001383842 MEDLINE

DOCUMENT NUMBER: 21141798 PubMed ID: 11246467

TITLE: Beta2-adrenergic receptor **allele** frequencies in the Quechua, a high altitude native population.

AUTHOR: Rupert J L; Monsalve M V; Devine D V; Hochachka P W

CORPORATE SOURCE: Department of Zoology, University of British Columbia, Vancouver, Canada.. rupert@zoology.ubc.ca

SOURCE: ANNALS OF HUMAN GENETICS, (2000 Mar) 64 (Pt 2) 135-43. Journal code: 0416661. ISSN: 0003-4800.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200107

ENTRY DATE: Entered STN: 20010709

Last Updated on STN: 20010709

Entered Medline: 20010705

AB The beta2-adrenergic receptor is involved in the control of numerous physiological processes and, as the primary catecholamine receptor in the lungs, is of particular importance in the regulation of pulmonary function. There are several polymorphic loci in the beta2-adrenergic receptor gene that have **alleles** that alter receptor function, including two (A/G46, G/C79) that increase **agonist** sensitivity. As such a phenotype may increase vaso and bronchial dilation, thereby facilitating air and blood flow through the lungs, we hypothesized that selection may have favoured these **alleles** in high altitude populations as part of an adaptive strategy to deal with the hypoxic conditions characteristic of such environments. We tested this hypothesis by determining the **allele** frequencies for these two **polymorphisms**, as well one additional missense **mutation** (C/T491) and two silent **mutations** (G/A252 and C/A523)

in 63 Quechua speaking natives from communities located between 3200 and 4200 m on the Peruvian altiplano. These frequencies were compared with those of two lowland populations, one native American (Na-Dene from the west coast of Canada) and one Caucasian of Western European descent. The Quechua manifest many of the pulmonary characteristics of high altitude populations and differences in **allele** frequencies between the Quechua and lowlanders could be indicative of a selective advantage conferred by certain genotypes in high altitude environments.

Allele frequencies varied between populations at some loci and patterns of linkage disequilibrium differed between the old-world and new-world samples; however, as these populations are not closely related, significant variation would be expected due to stochastic effects alone. Neither of the **alleles** associated with increased receptor sensitivity (A46, G79) was significantly over-represented in the Quechua compared with either lowland group. The Quechua were monomorphic for the C **allele** at base 79. This **variant** has been associated

with body mass index; however no clearly defined metabolic phenotype has been established. In addition, we sequenced the coding region of the gene in three unrelated Quechua to determine if there were any other **polymorphisms** common in this population. None were detected.

L8 ANSWER 16 OF 23 MEDLINE
ACCESSION NUMBER: 1998402654 MEDLINE
DOCUMENT NUMBER: 98402654 PubMed ID: 9731005
TITLE: beta2-Adrenergic receptor haplotypes in mild, moderate and fatal/near fatal asthma.
AUTHOR: Weir T D; Mallek N; Sandford A J; Bai T R; Awadh N;
Fitzgerald J M; Cockcroft D; James A; Liggett S B; Pare P D
CORPORATE SOURCE: Respiratory Health Network of Centres of Excellence,
University of British Columbia Pulmonary Research
Laboratory, St. Paul's Hospital, Vancouver, Canada.
CONTRACT NUMBER: HL45967 (NHLBI)
SOURCE: AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE,
(1998 Sep) 158 (3) 787-91.
Journal code: 9421642. ISSN: 1073-449X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981020
Last Updated on STN: 19981020
Entered Medline: 19981005

AB Excess beta2-**agonist** use in asthmatics has been associated with increased mortality and morbidity. The mechanisms responsible for these observations are unknown. We hypothesized that **polymorphisms** of the beta2-adrenergic receptor (beta2AR) at amino acid positions 16, 27, and 164, which are known to alter receptor functions in vitro, may predispose asthmatics to fatal/near-fatal asthma and/or modify asthma severity. In preliminary studies we found significant differences in **allele** frequencies due to ethnic background: Caucasian, Black, Asian Gly16 = 0.61, 0.50, 0.40 and Gln27 = 0.57, 0.73, 0.80, respectively. beta2AR genotyping was performed on DNA from Caucasians classified as nonasthmatic/nonatopic (n = 84), fatal/near-fatal asthmatics (n = 81) and mild/moderate asthmatics (n = 86). No **polymorphism** or haplotype was found to be associated with fatal/near-fatal asthma. However, the Gly16/Gln27 haplotype, which undergoes enhanced downregulation in vitro, was substantially more prevalent in moderate asthmatics than in mild asthmatics ($p = 0.003$, odds ratio = 3.1). We conclude that the beta2AR genotype is not a major determinant of fatal or near-fatal asthma. Furthermore, **allele** frequency variation among ethnic groups must be considered in clinical studies of beta2AR **polymorphisms** in asthma.

L8 ANSWER 17 OF 23 MEDLINE
ACCESSION NUMBER: 1999055070 MEDLINE
DOCUMENT NUMBER: 99055070 PubMed ID: 9817738
TITLE: The beta-adrenoceptor.
AUTHOR: Johnson M
CORPORATE SOURCE: Respiratory Therapeutic Development, Glaxo Wellcome
Research and Development, Uxbridge, Middlesex, United Kingdom.
SOURCE: AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE,
(1998 Nov) 158 (5 Pt 3) S146-53. Ref: 60
Journal code: 9421642. ISSN: 1073-449X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981221

AB The human beta-adrenoceptor is a member of the seven-transmembrane family of receptors, encoded by a gene on chromosome 5. beta-Adrenoceptors have been classified into beta1, beta2, and beta3 subgroups, with beta2-receptors being widely distributed in the respiratory tract, particularly in airway smooth muscle. Intracellular signaling following beta2-adrenoceptor activation is largely affected through a trimeric Gs protein coupled to adenylate cyclase. Cyclic AMP (cAMP) induces airway relaxation through phosphorylation of muscle regulatory proteins and attenuation of cellular Ca²⁺ concentrations. Alternative cAMP-independent pathways involving activation of membrane maxi-K⁺ channels and coupling through Gi to the MAP kinase system have also been described. Site-directed mutagenesis has identified Asp 113 and Ser 204/207 within the third and fourth membrane domains as the active site of the beta2-receptor, critical for beta2-agonist binding and activity. beta2-Agonists have been characterized as those that directly activate the receptor (**albuterol**), those that are taken up into a membrane depot (**formoterol**), and those that interact with a receptor-specific auxiliary binding site (**salmeterol**). These differences in mechanism of action are reflected in the kinetics of airway smooth muscle relaxation and bronchodilation in patients with asthma. beta-Adrenoceptor desensitization associated with beta2-agonist activation is a consequence of phosphorylation by beta-ARK and uncoupling of the receptor from Gs following beta-arrestin binding, of internalization and recycling of the receptor through processes of sequestration and resensitization and downregulation, modulated by an effect on receptor gene expression. The degree of receptor desensitization appears to differ, depending on the cell or tissue type, and is reflected in the different profiles of clinical tolerance to chronic beta2-agonist therapy. A number of polymorphisms of the beta2-receptor have been described that appear to alter the behavior of the receptor following agonist exposure. These include Arg-Gly 16, Glu-Gln 27, and Thr-Ile 164. The Gly 16 receptor downregulates to a greater extent and is associated with increased airway hyperreactivity, nocturnal symptoms, and more severe asthma. The Glu 27 form appears to protect against downregulation and is associated with less reactive airways. An individual can be homozygous or heterozygous for given polymorphisms, and large populations will have to be studied to determine their importance to the asthma phenotype.

L8 ANSWER 18 OF 23 MEDLINE
ACCESSION NUMBER: 1998064057 MEDLINE
DOCUMENT NUMBER: 98064057 PubMed ID: 9399946
TITLE: Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function.
AUTHOR: Large V; Hellstrom L; Reynisdottir S; Lonngqvist F; Eriksson P; Lannfelt L; Arner P
CORPORATE SOURCE: Department of Medicine, and Research Center, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden.
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1997 Dec 15) 100 (12) 3005-13.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 19980206

Entered Medline: 19980126

AB Catecholamines play a central role in the regulation of energy expenditure, in part by stimulating lipid mobilization through lipolysis in fat cells. The beta-2 adrenoceptor (BAR-2) is a major lipolytic receptor in human fat cells. To determine whether known **polymorphisms** in codons 16, 27, and 164 of this receptor play a role in obesity and subcutaneous adipocyte BAR-2 lipolytic function, we investigated a group of 140 women with a large variation in body fat mass. Only the **polymorphisms** in codons 16 and 27 were common in the study population. The Gln27Glu **polymorphism** was markedly associated with obesity with a relative risk for obesity of approximately 7 and an odds ratio of approximately 10. Homozygotes for Glu27 had an average fat mass excess of 20 kg and approximately 50% larger fat cells than controls. However, no significant association with changes in BAR-2 function was observed. The Arg16Gly **polymorphism** was associated with altered BAR-2 function with Gly16 carriers showing a fivefold increased **agonist** sensitivity and without any change in BAR-2 expression. However, it was not significantly linked with obesity. These findings suggest that genetic variability in the human BAR-2 gene could be of major importance for obesity, energy expenditure, and lipolytic BAR-2 function in adipose tissue, at least in women.

L8 ANSWER 19 OF 23 MEDLINE

ACCESSION NUMBER: 97470098 MEDLINE

DOCUMENT NUMBER: 97470098 PubMed ID: 9329515

TITLE: Association between beta 2-adrenoceptor **polymorphism** and susceptibility to bronchodilator desensitisation in moderately severe stable asthmatics.

COMMENT: Comment in: Lancet. 1998 Jan 3;351(9095):66-7

AUTHOR: Tan S; Hall I P; Dewar J; Dow E; Lipworth B

CORPORATE SOURCE: Department of Clinical Pharmacology and Therapeutics, University of Dundee, Ninewells Hospital and Medical School, UK.

SOURCE: LANCET, (1997 Oct 4) 350 (9083) 995-9.
Journal code: 2985213R. ISSN: 0140-6736.

PUB. COUNTRY: ENGLAND: United Kingdom
(CLINICAL TRIAL)

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RANDOMIZED CONTROLLED TRIAL)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 199711

ENTRY DATE: Entered STN: 19971224

Last Updated on STN: 19990129

Entered Medline: 19971106

AB BACKGROUND: In-vitro studies have suggested that **polymorphisms** of the beta 2-adrenoceptor may influence the desensitisation induced by beta 2-**agonists**. We investigated the influence of beta 2-AR **polymorphism** on the development of bronchodilator desensitisation in asthma patients. METHODS: We carried out an analysis of 22 moderately severe stable asthmatics, mean age 38 years, FEV1 63% of predicted and FEF25-75 38% of predicted, who received a median inhaled corticosteroid dose of 1000 micrograms/day. Patients were randomly assigned inhaled placebo or inhaled **formoterol** 24 micrograms bid for 4 weeks each in a crossover study. Bronchodilator dose-response curves were made at the end of each treatment period by use of cumulative doses of **formoterol** (6-108 micrograms) with FEV1 and FEF25-75 measured 30 min after each dose, and up to 6 h after the last dose. We calculated the degree of bronchodilator desensitisation by comparing the dose-response (for maximum and 6 h) after placebo with that after **formoterol**, and expressed this degree as a percentage of placebo response. Patients were divided into groups according to genotype at codon 16: homozygous Arg 16 (n = 4), heterozygous Arg 16/Gly 16 (n = 8), and homozygous Gly 16 (n = 10). At codon 27: homozygous Gln 27 (n = 5), heterozygous Gln 27/Glu 27

(n = 11), and homozygous Glu 27 (n = 6). FINDINGS: We found a significantly ($p < 0.05$) greater degree of bronchodilator desensitisation with homozygous Gly 16 than with homozygous Arg 16 for maximal FEV1 response: -8% (Arg 16) vs 46% (Gly 16); and for maximal FEF25-75 response: -32% (Arg 16) vs 74% (Gly 16; 95% CI 15-92% and 49-164%, respectively). Bronchodilator responses at 6 h were also significantly ($p < 0.05$) different for FEV1 and FEF25-75 when Arg 16 and Gly 16 were compared and values for heterozygous Arg 16/Gly 16 were intermediate. There was significantly greater desensitisation with Glu 27 than with Gln 27 for maximal FEF25-75 response: -7% (Gln 27) vs 68% (Glu 27), $p = 0.05$; and for 6 h FEF25-75 response: 43% (Gln 27) vs 93% (Glu 27), $p < 0.05$ (95% CI 2-147% and 5-94%, respectively). All patients who were homozygous Glu 27 were also homozygous Gly 16. INTERPRETATION: We have found preliminary evidence that beta 2-adrenoceptor **polymorphism** is associated with altered beta 2-adrenoceptor expression in asthma patients. The homozygous Gly-16 form was significantly more prone to bronchodilator desensitisation than Arg 16, with the influence of Gly 16 dominating over any putative protective effects of Glu 27.

L8 ANSWER 20 OF 23 MEDLINE
 ACCESSION NUMBER: 1998011963 MEDLINE
 DOCUMENT NUMBER: 98011963 PubMed ID: 9351598
 TITLE: **Polymorphisms** of the beta₂-adrenergic receptor and asthma.
 AUTHOR: Liggett S B
 CORPORATE SOURCE: Department of Medicine, University of Cincinnati College of Medicine, Ohio, USA.
 CONTRACT NUMBER: HL41496 (NHLBI)
 HL45967 (NHLBI)
 SOURCE: AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, (1997 Oct) 156 (4 Pt 2) S156-62.
 Journal code: 9421642. ISSN: 1073-449X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
 ENTRY MONTH: 199711
 ENTRY DATE: Entered STN: 19971224
 Last Updated on STN: 19971224
 Entered Medline: 19971106

AB Several missense **mutations** (**polymorphisms**) within the coding block of the **beta-adrenergic receptor** (**beta2AR**) gene on chromosome 5q31 have been identified in the human population. In studies utilizing site-directed mutagenesis and recombinant expression, three loci at amino acid positions 16, 27, and 164 have been found to significantly alter receptor function. The **Ile164** form displays altered coupling to adenylyl cyclase, the **Gly16** receptor displays enhanced **agonist**-promoted downregulation, and the **Glu27** form is resistant to downregulation. The frequencies of these various forms of the **beta2AR** are not different in asthmatics than in normal populations. However, given the importance of **beta2AR** in modulating lung function, studies have been carried out to determine if polymorphic forms may play roles in promoting asthmatic phenotypes, establishing bronchial hyperreactivity, or influencing the response to acute or chronic **beta-agonist** therapy. The results of case-control and family studies to date support these notions. Thus **beta2AR polymorphisms** act as disease modifiers in asthma and represent one of probably many genetic variables involved in the pathophysiology of asthma.

L8 ANSWER 21 OF 23 MEDLINE
 ACCESSION NUMBER: 96394533 MEDLINE
 DOCUMENT NUMBER: 96394533 PubMed ID: 8798639
 TITLE: Sustained activation of a G protein-coupled receptor via

"anchored" **agonist** binding. Molecular localization of the **salmeterol** exosite within the 2-adrenergic receptor.

AUTHOR: Green S A; Spasoff A P; Coleman R A; Johnson M; Liggett S B
CORPORATE SOURCE: Department of Medicine (Pulmonary), University of Cincinnati College of Medicine, Cincinnati, Ohio 45267, USA.
CONTRACT NUMBER: HL03346 (NHLBI)
HL45967 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Sep 27) 271 (39)
24029-35.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 20000303
Entered Medline: 19961118

AB An inherent therapeutic limitation of many G protein-coupled receptor **agonists** is a short duration of action due to rapid dissociation from receptors. **Salmeterol** is a modified **beta-adrenergic receptor** (betaAR) **agonist** that has a long duration of action at the beta2AR (but not the beta1AR) both in vitro and in vivo and that is persistent despite extensive washout of the **agonist**. It has been proposed that **salmeterol** binds not only to the active site of the beta2AR (localized to receptor transmembrane spanning domains (TMDs) 3 and 5) but also to another site (termed the "exosite") that anchors it to the receptor and provides for repetitive active-site binding events. To identify the location of this exosite, we used site-directed mutagenesis to replace beta2AR amino acids 149-173 (within TMD4) with beta1AR sequence. The resulting constructs were then expressed in COS-7 cells for radioligand binding studies. Using this approach, when this domain was replaced with the analogous beta1AR sequence, the ability of **salmeterol** to persist at the receptor under washout conditions was reduced by 67%. The results from more selective mutants (S-(149-166), S-(164-173), and S-(149-158)) indicated that a limited 10-amino acid region (beta2AR residues 149-158), localized at the interface of the cytoplasm and the transmembrane domain, contains a critical determinant for exosite binding. Whereas CHW cells stably expressing wild-type beta2AR displayed persistent **salmeterol**-promoted cAMP accumulation despite **agonist** washout, substitution of beta2AR residues 149-158 with beta1AR sequence resulted in a 56% attenuation of **salmeterol**-promoted cAMP accumulation under identical washout conditions. A reverse chimera was also studied, which consisted of a substitution of beta2AR residues 152-156 into the beta1AR. This substitution was found to confer exosite binding to the beta1AR. None of these **mutations** decreased the affinity of **salmeterol** for the receptor at the active site as assessed in competition binding studies. Anchored binding to this motif thus represents a novel mechanism by which **agonists** like **salmeterol** can repetitively activate receptors. Conceivably, with other G protein-coupled receptors that have similar motifs, anchored ligands can be designed to provide for long durations of action by this mechanism.

L8 ANSWER 22 OF 23 MEDLINE
ACCESSION NUMBER: 95221614 MEDLINE
DOCUMENT NUMBER: 95221614 PubMed ID: 7706471
TITLE: Genetic polymorphisms of the **beta 2-adrenergic receptor** in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype.

AUTHOR: Turki J; Pak J; Green S A; Martin R J; Liggett S B
CORPORATE SOURCE: Department of Medicine (Pulmonary), University of
Cincinnati College of Medicine, Ohio 45267-0564, USA.
CONTRACT NUMBER: HL-36577 (NHLBI)
HL-45967 (NHLBI)
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1995 Apr) 95 (4)
1635-41.
Journal code: 7802877. ISSN: 0021-9738.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950518
Last Updated on STN: 19970203
Entered Medline: 19950509

AB Nocturnal asthma represents a unique subset of patients with asthma who experience worsening symptoms and airflow obstruction at night. The basis for this phenotype of asthma is not known, but **beta 2-adrenergic receptors** (**beta 2AR**) are known to downregulate overnight in nocturnal asthmatics but not normal subjects or nonnocturnal asthmatics. We have recently delineated three polymorphic loci within the coding block of the beta 2AR which alter amino acids at positions 16, 27, and 164 and impart specific biochemical and pharmacologic phenotypes to the receptor. In site-directed mutagenesis/recombinant expression studies we have found that glycine at position 16 (Gly16) imparts an accelerated **agonist**-promoted downregulation of beta 2AR as compared to arginine at this position (Arg16). We hypothesized that Gly16 might be overrepresented in nocturnal asthmatics and thus determined the beta 2AR genotypes of two well-defined asthmatic cohorts: 23 nocturnal asthmatics with 34 +/- 2% nocturnal depression of peak expiratory flow rates, and 22 nonnocturnal asthmatics with virtually no such depression (2.3 +/- 0.8%). The frequency of the Gly16 **allele** was 80.4% in the nocturnal group as compared to 52.2% in the nonnocturnal group, while the Arg16 **allele** was present in 19.6 and 47.8%, respectively. This overrepresentation of the Gly16 **allele** in nocturnal asthma was significant at P = 0.007 with an odds ratio of having nocturnal asthma and the Gly16 **polymorphism** being 3.8. Comparisons of the two cohorts as to homozygosity for Gly16, homozygosity for Arg16, or heterozygosity were also consistent with segregation of Gly16 with nocturnal asthma. There was no difference in the frequency of **polymorphisms** at loci 27 (Gln27 or Glu27) and 164 (Thr164 or Ile164) between the two groups. Thus the Gly16 **polymorphism** of the beta 2AR, which imparts an enhanced downregulation of receptor number, is overrepresented in nocturnal asthma and appears to be an important genetic factor in the expression of this asthmatic phenotype.

L8 ANSWER 23 OF 23 MEDLINE
ACCESSION NUMBER: 94043092 MEDLINE
DOCUMENT NUMBER: 94043092 PubMed ID: 7901205
TITLE: A **polymorphism** of the human **beta 2-adrenergic receptor** within the fourth transmembrane domain alters ligand binding and functional properties of the receptor.
AUTHOR: Green S A; Cole G; Jacinto M; Innis M; Liggett S B
CORPORATE SOURCE: Department of Medicine (Pulmonary), University of Cincinnati College of Medicine, Ohio 45267-0564.
CONTRACT NUMBER: RO1-HL45967 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Nov 5) 268 (31)
23116-21.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19980206
Entered Medline: 19931129

AB We have recently identified several naturally occurring **variants** of the human **beta** 2-adrenergic receptor (**beta** 2AR). One of these **polymorphisms**, which is relatively uncommon, is a **mutation** occurring in the fourth transmembrane spanning domain, with Ile substituted for Thr at amino acid 164 within the proposed ligand binding pocket. This **mutation** is adjacent to Ser165 which has been predicted to interact with the beta-carbon hydroxyl group of adrenergic ligands. To determine the functional significance of this **variant**, we constructed by site-directed techniques a mutated beta 2AR (**Ile164**) with this substitution and expressed it in CHW-1102 cells. In the presence of guanine nucleotide, **Ile164** displayed a lower binding affinity for epinephrine as compared with the wild-type beta 2AR ($K_i = 1450 +/- 79$ versus $368 +/- 39$ nM; $p < 0.001$). A similarly decreased affinity was found with the catecholamines isoproterenol and norepinephrine, but not with dobutamine or dopamine which lack hydroxyl groups on their beta-carbons. In addition, antagonists without aromatic ring polar substituents displayed a decreased affinity for the mutated receptor. In **agonist** competition experiments conducted in the absence of guanine nucleotide, **Ile164** failed to exhibit detectable high affinity binding, suggesting an impairment in the formation of the **agonist**-receptor-Gs complex. Consistent with this finding, functional coupling to Gs as determined in adenylyl cyclase assays was significantly (approximately 50%) depressed with **Ile164** under both basal and **agonist**-stimulated conditions. beta 2AR sequestration, which is also triggered by **agonist** binding, was also found to be approximately 65% reduced in the **Ile164** **polymorphism**. This study represents the first characterization of a naturally occurring **mutation** of a human adrenergic receptor. Our findings generally support the hypothesized role of this region of the receptor for ligand binding and receptor activation, as well as for establishing critical interactions for overall receptor conformation.

L Number	Hits	Search Text	DB	Time stamp
1	1	"6586183"	USPAT; US-PGPUB; DERWENT	2003/07/14 09:17
2	37	"0106910"	USPAT; US-PGPUB; DERWENT	2003/07/14 09:18
3	0	variation same drug same response same polymorphisms same receptor	USPAT; US-PGPUB; DERWENT	2003/07/14 09:19
4	0	variation same drug same response same polymorphisms	USPAT; US-PGPUB; DERWENT	2003/07/14 09:19
5	6	ile164	USPAT; US-PGPUB; DERWENT	2003/07/14 09:20
6	73	"0010125"	USPAT; US-PGPUB; DERWENT	2003/07/14 09:20
7	2	"2000010125"	USPAT; US-PGPUB; DERWENT	2003/07/14 09:33
8	27	liggett-s\$.in.	USPAT; US-PGPUB; DERWENT	2003/07/14 09:34
9	1	1999-277657.NRAN.	DERWENT	2003/07/14 09:34
10	1	2001-182691.NRAN.	DERWENT	2003/07/14 09:53